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Original Article

# Analyses of the relationships among biofilm activity, antibiotic resistance and expressions of *SHV, TEM, CTX* and *IntI* in *Klebsiella pneumoniae*

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**Abstract**

**Aim:** *Klebsiella pneumoniae* as an opportunistic pathogen is responsible for nosocomial, urinary tract infections, pneumonia, bacteremia, liver abscesses, and respiratory and blood infections in patients.
The aim of our study is to examine the antibiotic sensitivity, expression levels of *IntI*, *TEM*, *SHV* and *CTX* genes, which play a role in multidrug resistance, biofilm formation and even the relationships among them.
**Materials and Methods:** Identification was implemented by carrying out the VITEK2 system and phenotypic confirmation of the ESBL producing isolates by using a combined disc test. Antibiotic sensitivity was implemented by disc diffusion method and microdilution methods toward various antibiotics. Furthermore, biofilm formation was examined through microtitration plate method.
**Results:** The expressions of *IntI*, *TEM*, *SHV* and *CTX* were implemented in qRT-PCR. 19 were susceptible to all antibiotics, 17 were ESBL (+) and 16 were carbapenem-resistant among 52. The expression of these genes was upregulated in ESBL (+) strains and produced biofilm and the expression of these genes was upregulated, too.
**Conclusion:** The results of the study revealed the potential role of the mentioned genes in biofilm formation and antibiotic resistance, enabling the development of new drugs. Further studies will be essential so as to determine when or how these systems are included in antibiotic resistance and biofilm formation.

**Keywords:** *Klebsiella pneumoniae*; biofilm; *IntI; TEM; SHV; CTX*

## INTRODUCTION

Biofilm is an ability of bacteria to provide communication in each other. A biofilm is embedded in extracellular polymeric substances (EPS) matrix which are formed by bacteria. This matrix allows the bacterium to stick to the surface (1). Several bacteria regulate gene expression via intercellular communication which is named as quorum sensing (QS) (2). Both Gram-positive and Gram-negative bacteria can form biofilms. Amongst these bacteria are: *Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus viridans, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis and Pseudomonas aeruginosa* (3).

*Klebsiella pneumoniae* is an encapsulated, nonmotile, facultative anaerobic, catalase positive, oxidase negative, rod-shaped member of the Enterobacterales family and not only a Gram-negative opportunistic bacteria in the natural area but also a pathogen causing health care associated infections that primarily affect immunocompromised patients (4-6). It is due to some by hospital-based causes in the respiratory tract, urinary tract infections, surgical wounds and septicaemia. *K. pneumoniae* is featured by its capability to generate several different kinds of virulent factors such as fimbriae, antiphagocytic capsule, adherence properties, lipopolysaccharides, membrane transporters, siderophores, and efflux, leading to high mortality and morbidity rates (7). Moreover, bacteria cause

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multidrug-resistance to antibiotics, which are extended-spectrum lactamases (ESBLs) and even responsible for health care associated infections (8). Antimicrobial resistance mechanism can be procured by a genetic mutation, horizontal transfer or vertical transfer via mobile genetic parts such as plasmid and transposons (integron) also have been authenticated the major role in spreading of the resistant genes (9).

*K. pneumoniae* can show a high resistance to third-generation cephalosporins by acquiring the plasmids harboring genes encoding ESBLs (10). Most ESBL enzymes in *K. pneumoniae* originate from two classical enzyme types *TEM* and *SHV* encoded by the plasmid. Transposable elements located in plasmids can be redivided into two fundamental groups, the superintegrons and the antibiotic resistance integrons (ARIs). Among various classes of ARIs, classes 1, 2, and 3 have been implied with multiple antibiotic resistance (MAR) phenotypes and are characterized depending on their own integrase (*IntI*) encoded by the integron (11). As a result, supply of these plasmids by the bacterium increased its virulence potential. Furthermore, *Klebsiella pneumoniae* strains forming *CTX*-M type have risen (12). The fast spread of *CTX*-M enzymes is promoted by plasmids, transposons and integron gene cassettes (13).

**The purpose of this study was to evaluate**

1. The relationships between amid biofilm formation and antibiotic resistance

2. The relationships between amid biofilm formation and the expressions of *SHV*, *TEM*, *CTX*,

3. The relationships between amid biofilm formations and the expression of *IntI*,

4. The relationships between amid the expressions of *SHV*, *TEM*, *CTX* and *IntI*.

The purpose of this study was to evaluate the relationships between amid biofilm formation and antibiotic resistance. Also evaluate the relationship between amid biofilm formation and expressions of *SHV-TEM-CTX*, *IntI* and *SHV-TEM-CTX-IntI*.

## MATERIALS and METHODS

We divided our isolates (52 isolates) into three groups: a) All antibiotic sensitive b) ESBLs positive c) carbapenem-resistant.

### Bacterial strains, Growth Conditions

In the 2013-2017 periods, 52 isolates were collected from tracheal aspirate and urine in Amasya Sabuncuoğlu Serefeddin Training and Research Hospital. A sterile swab was used to take sample from patients. The samples were immediately cultured on eosin methylene blue agar (EMB) (RTA, Turkey), 5% sheep blood agar (RTA, Turkey), and incubated overnight at 37°C. Any unusual colony was then subcultured and made pure. All bacterial strains were collected at 20°C in brain heart infusion broth (BHI) (Merck, Germany) including %15 glycerol (Sigma, UK). Bacteria were subcultured in Tryptic Soy Agar (TSA) (Merck, Germany) and Tryptic Soy Broth (TSB) (Merck, Germany) at 37°C.

### Antibiotic Susceptibility Test

Disc Diffusion Assay: Antimicrobial sensitivity testing of the isolates was executed by means of the standard disc diffusion method as advised by the Clinical and Laboratory Standards Institute (14). American Typing Collection (ATCC 25922) of *Escherichia coli* was utilized as a control strain. The antibiotic susceptibility test was executed by disc diffusion method with commercially accessible discs (Bioanalyse, Turkey). Susceptibilities of isolates were assessed against cefazolin (CZ, 30 µg), cefuroxime (CXM, 30 µg), ceftriaxone (CRO 30 µg), cefepime (FEB, 5 µg), piperacilin-tazobactam (TPZ, 10/100 µg), imipenem (IPM, 10 µg), meropenem (MEM, 10 µg), gentamicin (CN, 30 µg), amikacin (AK, 30), ciprofloxacin (CIP, 5 µg), and levofloxacin (LEV, 5 µg). The diameter of the inhibition zone for each antibiotic was calculated and interpreted as resistant, intermediate susceptible or susceptible according to CLSI criteria (14).

Broth microdilution assay: For antibiotics MICs (minimum inhibition concentration) were specified following CLSI instructions using the broth microdilution method with cation-adjusted Mueller–Hinton broth (Sigma). Organisms were inoculated at 5x104 cells per well. The MIC was described as the lowest concentration that inhibited seeable growth after incubation at 37°C for 18 hours. *Escherichia coli* ATCC 25922 was used as a control strain for the detection of antibacterial susceptibility. After overnight incubation, MICs was interpreted as the lowest concentration inhibiting seeable growth (14).

Biofilm Formation Assay: The capability of *K. pneumoniae* to produce biofilms in vitro was determined by means of the microtiter plate assay according to described methods of (15). Firstly, *K. pneumoniae* isolates ($10^8$ CFU/mL) were inoculated into Tryptic Soy Broth (TSB) (Merck, Germany) with 2% glucose as a carbon source. Polystyrene microtitre 96-well plates were made in triplicate and incubated at 37°C for 24 h. Later 24 h incubation period, a medium was ejected from wells and then plate wells were washed thrice with phosphate buffer saline (PBS). Plates were air dried for 30 mins and each hole was stained with 1% crystal violet solution. After staining, plates were washed at least three times with sterile distilled water. The quantitative study of biofilm formation was implemented by adding %95 ethanol to destain the wells. Cell turbidity was measured by means of a microtiter plate reader (Multiskan GO, Thermo Scientific, Germany) at 570 nm ($OD_{570}$). *E. coli* ATCC 25922 was utilized as negative control and P. aeruginosa ATCC 15692 was utilized as positive control.

### Molecular analyses

RNA isolation and cDNA Synthesis: 24 hours cultures were grown in TSB in the incubator and allow reaching mid-logarithmic phase (OD600 of 0.7-1) at 37°C. Total RNA was extracted using the GeneJET RNA Purification

Kit (Thermo Scientific, USA) by the producer's guidelines. The concentration and purity of total RNA samples were detected by means of a spectrophotometer. The cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) from 100 ng of total RNA with Oligo $(dT)_{18}$ primers following the producer's instructions. cDNA was reserved at -20°C until required.

### Real-Time PCR

The relative expression of *IntI*, *TEM*, *SHV* and *CTX* genes was implemented in PikoReal™ Real-Time PCR System (Thermo Scientific, USA) with Maxima SYBR Green qPCR Master Mix (2X) (Thermo Scientific, USA) kit according to the producer's instructions. The primers utilized for qRT-PCR were demonstrated in Table 1. Shortly, the reaction mixture was comprised of 5 µl of Maxima SYBR Green qPCR Master Mix (2X), 0.5 µl of forward primer (0.5 µM), 0.5 µl of reverse primer (0.5 µM), 1 µl of cDNA, and 3 µl of nuclease-free water in 10 µl reaction volume. Melting curve analyses were implemented to ensure specificity. Fold change of each gene was measured using the ΔΔCT method (16). Fold change in gene expression was determined with the normalisation of every gene to the 16S rRNA inner control and relative to *K. pneumoniae* ATCC 700721 as a calibrator. Moreover, Negative Template Control (NTC) was used to detect contamination.

Biofilm formation and expression profiles were assessed by using Bio-Array Resource (BAR) HeatMapper Plus Tool website (http://bar.utoronto.ca/). Results were charted and formatted to a color scale again to visualize expression in terms of percentage (Figure 1).

**Table 1. Group-specific primers used for the assays**

| Primer | Primer Sequence 5' 3' | Reference |
|---|---|---|
| ***CTX*-M-Y1-F** | ATGGTTAAAAAATCACTGCGC | |
| ***CTX-M-Y1-R*** | TTACAAACCGTCGGTGACGAT | |
| **bla*TEM*-F** | TAACCATGAGTGATAACACT | Dallenne et al., 2010 |
| **bla*TEM*-R** | CCGATCGTTGTCAGAAGTAA | |
| **bla*SHV*-F** | ACTGCCTTTTTGCGCCAGAT | |
| **bla*SHV*-R** | CAGTTCCGTTTCCCAGCGGT | |
| **IntI-F** | CCTCCCGCACGATGATC | Bass et al., 1999 |
| **IntI-R** | TCCACGCATCGTCAGGC | |

## RESULTS

A collection of 52 *K. pneumoniae* was gathered from intensive care unit (ICU) patients in Amasya University Sabuncuoğlu Serefeddin Education and Research Hospital, Turkey. Most *K. pneumoniae* isolates were taken from urine and tracheal aspirate. These isolates were characterized according to biofilm formation, antibiotic susceptibility and *IntI*, *TEM*, *SHV* and *CTX* expression profiles.

### Disc diffusion

We classified clinical isolates of *K. pneumoniae* in accordance with different antibiotics. Results indicated that 19 were susceptible to all antibiotics (37%). 17 (33%) were ESBL positive and 16 were carbapenem-resistant (30%) among 52 isolates according to CLSI, 2015 (Table 3).

### Susceptibilities of *K. pneumoniae*

A sensitivity pattern of *K. pneumoniae* to various antibiotics was shown in Table 2. The MIC criteria for resistance and sensitivity to each antibiotic were determined according to the suggestiond of the CLSI (2015) criteria. Table 4, 5, 6 showed the lowest concentration of antibacterial agents, preventing the development of 50% and 90 % of all of the bacterial isolates which were tested ($MIC_{50}$ and $MIC_{90}$). ESBLs positive strains were the highest resistant among all tested antibiotic agents such as cefazolin, cefepime, cefuroxime, ceftriaxone, piperacillin-tazobactam. Among these group antibiotics, cefepime and cefazolin were the highest activity $MIC_{50}$ and $MIC_{90}$ (≥ 64 µg/ml). In contrast, β-lactamase inhibitor group (piperacillin/tazobactam) was the lowest activity $MIC_{50}$ and $MIC_{90}$ (4 µg/ml and 16 µg/ml), respectively. Among all antibiotics groups, levofloxacin and ciprofloxacin belonging to fluoroquinolone group antibiotics indicated the highest antimicrobial activity in terms of MIC50 and MIC90 values (≤ 0.25 and 2 µg/ml) (Table 4, 5, 6). Moreover, in carbapenem-resistant isolates, antibiotics cefazolin, cefuroxime and cefepime were the highest activity MIC50 and MIC90 (≥ 64 µg/ml). Among all antibiotics groups, levofloxacin belonging to fluoroquinolone group antibiotics indicated the highest antimicrobial activity in terms of MIC50 and MIC90 values (≤ 0.25 and 8 µg/ml) (Tablo 4, 5, 6).

**Table 2. Sensitivity pattern of K. pneumoniae to different antibiotics**

| | BiofilmFormation | |
|---|---|---|
| **All isolates (n:52)** | **Positive (n)** | **Negative (n)** |
| **All antibiotic-sensitive (19)** | 13 | 6 |
| **ESBL positive(17)** | 17 | - |
| **Carbapenam-resistant(16)** | 7 | 9 |

**Table 3. Classification of clinical isolates of K. pneumoniae according to antibiotic variables**

| **Allisolates (n=52)** | **Isolates n (%)** |
|---|---|
| **All antibiotic-sensitive** | 19 (37%) |
| **ESBLspositive** | 17 (33%) |
| **Carbapenem-resistant** | 16 (30%) |
| **Susceptibility interpreted according to current CLSI break points (CLSI, 2015)** | |

**Table 4. MIC values of all antibiotic sensitive 19 strains K. pneumoniae (µg/mL)**

| Antibiotics | (Allisolates n=19) | | |
|---|---|---|---|
| | Range | $MIC_{50}$ | $MIC_{90}$ |
| CZ | ≤1 - ≥64 | 2 | 4 |
| CXM | ≤1 - ≥64 | 2 | 4 |
| CRO | ≤0.25 - ≥64 | ≤0.25 | ≤0.25 |
| FEP | ≤0.25 - ≥64 | 2 | 4 |
| TPZ | ≤1 - 64 | 4 | 4 |
| IPM | ≤0.25 - 64 | ≤0.25 | ≤0.25 |
| MEM | ≤0.25 - 64 | ≤0.25 | 1 |
| CN | ≤0.25 - 32 | 1 | 1 |
| AK | ≤0.25 - 32 | 2 | 4 |
| CIP | ≤0.25 - 16 | ≤0.25 | ≤0.25 |
| LEV | ≤0.25 - 16 | ≤0.25 | ≤0.25 |

CZ, cefazolin; CXM, cefuroxime; CRO, ceftriaxone; FEP, cefepime; TPZ, piperacillin-tazobactam; IPM, imipenem; MEM, meropenem; CN, gentamicin; AK, amikacin; CIP, ciprofloxacin; LEV, levofloxacin. MIC, minimum inhibitoryconcentration; MIC50/90, concentration at whichpreventsgrowth of 50% and 90% of theisolates.

**Table 5. Antimicrobial activities of 17 strains of ESBLs positive K. Pneumoniae (µg/mL)**

| Antibiotics | (Allisolates n=17) | | |
|---|---|---|---|
| | Range | $MIC_{50}$ | $MIC_{90}$ |
| CZ | ≤1 -≥64 | ≥64 | ≥64 |
| CXM | ≤1 -≥64 | 32 | ≥64 |
| CRO | ≤0,25 -≥64 | 8 | 16 |
| FEP | ≤0.25 -≥64 | ≥64 | ≥64 |
| TPZ | ≤1 - 64 | 4 | 16 |
| IPM | ≤0.25 - 64 | ≤0.25 | ≤0.25 |
| MEM | ≤0.25 - 64 | ≤0.25 | 1 |
| CN | ≤0.25 - 32 | 1 | 8 |
| AK | ≤0.25 - 32 | 2 | 4 |
| CIP | ≤0.25 - 16 | ≤0.25 | 2 |
| LEV | ≤0.25 - 16 | ≤0.25 | 2 |

CZ, cefazolin; CXM, cefuroxime; CRO, ceftriaxone; FEP, cefepime; TPZ, piperacillin-tazobactam; IPM, imipenem; MEM, meropenem; CN, gentamicin; AK, amikacin; CIP, ciprofloxacin; LEV, levofloxacin. MIC, minimum inhibitoryconcentration; MIC50/90, concentration at whichpreventsgrowth of 50% and 90% of theisolates.

**Table 6. Antimicrobial activities of 16 strains of Carbapenem resistant K. Pneumoniae (µg/mL)**

| Antibiotics | (Allisolates n=17) | | |
|---|---|---|---|
| | Range | $MIC_{50}$ | $MIC_{90}$ |
| CZ | ≤1 -≥64 | ≥64 | ≥64 |
| CXM | ≤1 -≥64 | ≥64 | ≥64 |
| CRO | ≤0.25 -≥64 | 32 | ≥64 |
| FEP | ≤0.25 -≥64 | ≥64 | ≥64 |
| TPZ | ≤1 -64 | 32 | 64 |
| IPM | ≤0.25 -64 | 32 | 64 |
| MEM | ≤0.25 -64 | 32 | 64 |
| CN | ≤0.25 - 32 | 8 | 32 |
| AK | ≤0.25 - 32 | 4 | 4 |
| CIP | ≤0.25 - 16 | 4 | 4 |
| LEV | ≤0.25 - 16 | ≤0.25 | 8 |

CZ, cefazolin; CXM, cefuroxime; CRO, ceftriaxone; FEP, cefepime; TPZ, piperacillin-tazobactam; IPM, imipenem; MEM, meropenem; CN, gentamicin; AK, amikacin; CIP, ciprofloxacin; LEV, levofloxacin. MIC, minimum inhibitoryconcentration; MIC50/90, concentration at whichpreventsgrowth of 50% and 90% of theisolates.

### Biofilm formation

19 were susceptible to all antibiotics, 17 were ESBL positive and 16 were carbapenem-resistant among 52. Furthermore, 13 of the 19 susceptiple to all antibiotics strains, the entire ESBL positive and 7 of the 16 carbapenem-resistant strains formed biofilm (Table 2). Besides, Figure 1 indicated that the highest result of biofilm formation was acquired from ESBL positive isolates (Figure 1).

### Expression profiles

We detected the expressions of these four genes in all antibiotic resistance, ESBL positive and carbapenem-resistant isolates. *IntI* expression of 2 susceptible to all antibiotics and 15 ESBL (+) and also 12 carbapenem-resistant were upregulated. *TEM* expression of 3 susceptible to all antibiotics and 7 ESBL (+) and 8 carbapenem-resistant were upregulated. *SHV* expression of 18 susceptible to all antibiotics, 15 ESBL (+) and 15 carbapenem-resistant were upregulated. *CTX* expression of all susceptible was down regulated whereas 15 ESBL (+) and 2 carbapenem-resistant were upregulated.

Genes related to beta-lactamase group (*TEM*, *CTX*, *SHV*) upregulated in all antibiotic resistance. In addition to these genes, *Int1* gene also increased in all antibiotic resistant isolates. Moreover, expression profile order in all antibiotic-resistant isolates was *Int1*>*TEM*>*CTX*>*SHV*. In addition, the results of ESBL positive and carbapenem-resistant isolates were varying results (Figure 1).

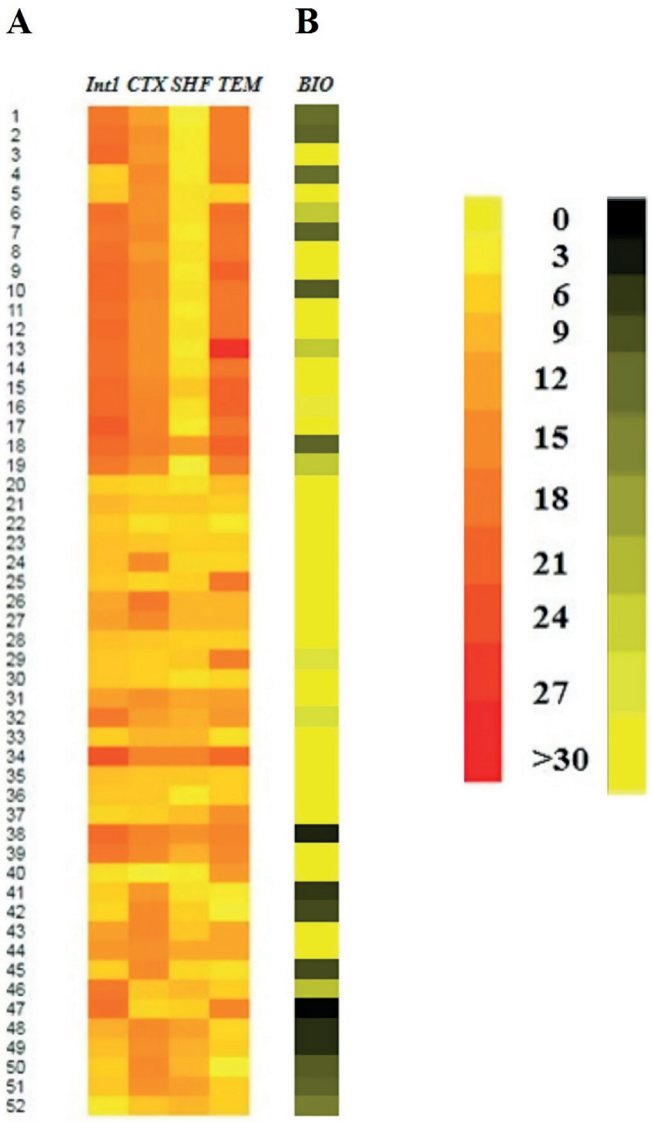


**Figure 1.** Expression profiles of *IntI*, *TEM*, *SHV* and *CTX*. Antibiotic resistance (19), ESBL positive (19) carbapenem (16) isolates were color-coded (yellow and red for relatively lower or higher expression, respectively) as signified in the scale bar. B) Biofilm formation results.1-19 isolates were all antibiotics sensitive; 26-36 isolates were ESBL positive, and 37-52 isolates were Carbapenem-resistant.

## DISCUSSION

Today, ESBL- and carbapenemase-synthesise *K. pneumoniae* strains have turned into a major issue aorund the world (17, 18).Therefore, there are many studies related to figure out the mechanisms of antibiotic resistance and even prevent this. In our study, 52 *K. pneumoniae* isolates were gathered from intensive care unit (ICU) patients in Amasya. Results showed that 19 of them were susceptible to all antibiotics, 17 of them were ESBL positive and 16 of them were carbapenem-resistant. Also, 13 of the sensitive strains, all of the ESBL strains and 7 of the carbapenem-resistant strains formed biofilm. ESBLs positive strains were the highest resistant among all tested antibiotic agents such as cefazolin, cefepime, cefuroxime, ceftriaxone, piperacillin-tazobactam. Previous research have revealed that the rate of ESBL- and carbapenemase-synthesise *K. pneumoniae* isolates can be varied from 19% to 60% in different countries (19-22). In our study, we detected that 33% of collected isolates were ESBL-producers, 30% of them were carbapenem-resistant while 37% were all antibiotic resistant. Different results could be unbalanced use of this class of antibiotics against diseases caused by *K. pneumoniae*. There are many studies reporting resistance of this bacteria against third-generation cephalosporins, carbapenems, fluoroquinolones, and aminoglycosides (17, 23, 24).

Biofilm protects the pathogen from the host immune responses besides from antibiotics, developing its endurance on epithelial tissues and medical device surfaces (7). Therefore, biofilm formation is a significant virulence agent in many species (25). It has been reported that microbial biofilm formation and development have a major role in *Klebsiella* pathogenicity (10, 26, 27). Our experimental groups can be divided into three classes: all antibiotic-sensitive, ESBL-producing, and carbapenem-resistant. We reported that especially, ESBL producing isolates (33%) produced biofilm. Therefore, it was concluded that biofilm formation could be directly related to antibiotic resistance in *K. pneumoniae* insulated from intensive care unit patiences. Concordant with these results, (28) showed important relationships between biofilm and ESBL formation in 150 *K. pneumoniae* strains, obtained from sputum and urine. Afterwards, (10) and (29) confirmed that ESBL producer strains with more biofilm than non-ESBL producer.

Some virulence-related genes found in *K. pneumoniae* are included in biofilm formation, too (3, 7). Moreover, (30) also concluded that marker genes on plasmids had a significant role about both biofilm cells' resistance to antibiotics and mature biofilms' information. To support this, we detected the upregulation of *IntI* expression in 2 sensitive to all antibiotics and 15 ESBL (+) and also 12 carbapenem-resistant isolates. Thus, we could say that there were also the relationships between *IntI* expression and antibiotic resistance. Previous studies were concluded that integrons have a significant act in the mechanisms of antimicrobial drug-resistant via the integron-encoded integrase (9, 31). In addition to *IntI* expression, we also analyze the expressions of *TEM*, *SHV* and even *CTX*. The expressions of *SHV* and *CTX* in ESBL (+) and carbapenem-resistant isolates were upregulated. (32) reported asimilar result. They found that 4 isolates of 15 *Klebsiella* spp. (26.26%) had a *SHV* gene.

In addition, (33) concluded that *TEM* and *SHV* were 93.75% and 87.5 % in *K. pneumoniae*, respectively. (34) demonstrated a different point related to *CTX*-M-2-including complex class 1 integrons. They reported that these integrons were not located in plasmids, therefore, the integration properties into the bacterial chromosome also provide the maintenance among the strains in the environment. On the other hand, (35) suggested that

*CTX*-M-encoding genes were mainly settled on plasmids, and plasmid-mediated transmission of bla*CTX*-M genes in Enterobacteriaceae including several mobile genetic factors has been characterized. Supporting our data, (36) also indicated plasmids carrying ESBL encoding genes and even integron provide antibiotic resistance has in nosocomial isolates of *K. pneumoniae*. *TEM* expression of 3 sensitive to all antibiotics and 7 ESBL (+) and 8 carbapenem-resistant were also upregulated in this presented study. Reports suggested that local antibiotic usage might play an active role in promoting the selection of point mutations in bla*TEM*-type genes (37). Furthermore, different studies also showed similar results related to these genes in *Klebsiella* spp. (20, 32, 33, 37-39).

The upward rate of *K. pneumoniae* strains resistant to multiple antibiotics is a worldwide, public health issue. Hence, ESBL-producing organisms must be quickly identified in terms of microbiological and molecular analysis lest suitable antibiotic, usage and infection control systems could be applied (7, 13). The present work indicated that the relationships among biofilm formation, antibiotic resistance and expressions of *IntI*, *SHV*, *TEM*, *CTX*. This study may help to figure out the antibiotic resistance mechanisms in *K. pneumoniae* and to control of resistant infections in the hospital environment.

## CONCLUSION

The present work indicated that the relationships among biofilm formation, antibiotic resistance and expressions of *IntI, SHV, TEM, CTX*. This study may help to figure out the antibiotic resistance mechanisms in K. *pneumoniae* and to control of resistant infections in the hospital environment.

*Competing interests: The authors declare that they have no competing interest.*

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